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Diversity of bacteria producing pigmented colonies in aerosol, snow and soil samples from remote glacial areas (Antarctica, Alps and Andes)

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Abstract

Four different communities and one culture of pigmented microbial assemblages were obtained by incubation in mineral medium of samples collected from high elevation snow in the Alps (Mt. Blanc area) and the Andes (Nevado Illimani summit, Bolivia), from Antarctic aerosol (French station Dumont d'Urville) and a maritime Antarctic soil (King George Island, South Shetlands, Uruguay Station Artigas). Molecular analysis of more than 200 16S rRNA gene sequences showed that all cultured cells belong to the *Bacteria* domain. The phylogenetic comparison with the currently available rDNA database allowed the identification of sequences belonging to *Proteobacteria* (*Alpha-, Beta- and Gamma-proteobacteria*), *Actinobacteria* and *Bacteroidetes* phyla. The Andes snow culture was the richest in bacterial diversity (eight microorganisms identified) and the maritime Antarctic soil the poorest (only one). Snow samples from Col du midi (Alps) and the Andes shared the highest number of identified microorganisms (*Agrobacterium*, *Limnobacter*, *Aquiflexus* and two uncultured *Alphaproteobacteria* clones). These two sampling sites also shared four sequences with the Antarctic aerosol sample (*Limnobacter*, *Pseudonocardia* and an uncultured *Alphaproteobacteria* clone). The only microorganism identified in the maritime Antarctica soil (*Brevundimonas* sp.) was also detected in the Antarctic aerosol. The two snow samples from the Alps only shared one common microorganism. Most of the identified microorganisms have been detected previously in cold environments (*Dietzia kujamenisi*, *Pseudonocardia Antarctica*, *Hydrogenophaga palleronii* and *Brebundimonas* sp.), marine sediments (*Aquiflexus balticus*, *Pseudomonas pseudoalkaligenes*, *Pseudomonas* sp. and one uncultured *Alphaproteobacteria*), and soils and rocks (*Pseudonocardia* sp., *Agrobactrium* sp., *Limnobacter* sp. and two uncultured *Alphaproteobacetria* clones). Air current dispersal is the best model to explain the presence of very specific microorganisms, like those used in this work, in very distant environments. In addition these microorganisms have to be resistant to extreme conditions and able to grow in oligotrophic environments. Considering the habitats in which they have been identified, the presence

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of pigments must be related with their ability to resist high doses of radiation.

1 Introduction

Long distance dispersal of biological particles produced by atmospheric circulation, ocean currents, birds, fish, mammals and human vectors, has been known for a long time (Gislén, 1948; Gregory, 1967; Schnell and Vali, 1972; Marshall 1996a, b; Vincent, 2000). Microorganisms have small dimensions (usually less than 5 μm in size) and therefore are easily transported by air masses. Eventually, these wind-born particles are deposited on the ground and/or snow/ice, on very high mountains and polar regions, respectively, in either dry or wet form (Chalmers et al., 1996). It has been proposed that microorganisms living in hot and/or cold terrestrial deserts are especially important for the dispersion of microorganisms due to their special adaptations to extreme and variable conditions (temperature, light intensity, spectral quality, water, desiccation, etc.) (Flechtner, 1999; Van Thielen and Garbary, 1999; Garty, 1999; Elster and Benson, 2004).

Living microorganisms have been collected in the stratosphere (Imshenetsky et al., 1978), and there are several reports supporting the idea that microorganisms can live and reproduce on airborne particles (Dimmick et al., 1979). It has been also shown that microorganisms can actively grow and reproduce at temperatures near or below 0°C, in cloud droplets collected at high altitudes (Sattler et al., 2001).

Both, the viable and dead cells, or their remnants, stored in the ice of continental icecaps and mountain glaciers have the potential to represent a historical record of the recent evolution of microbial life, as well as a record of the Earth's changing climate. In the wake of the recent discovery of the sub-glacial Antarctic Lake Vostok (Priscu et al., 1999; Siegert et al., 2001), and the possibility of recovering water samples containing fossil living microorganisms, there is a growing interest in investigating the transport of living organisms via air over large distances, e.g. cold glacial regions.

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l'Environnement du CNRS, St Martin d'Heres, France, the composition of micro-autotrophs (cyanobacteria and algae), micro-fungi (hyphae and spores), bacteria (rods, cocci and pigmented bacterial colonies), yeast and plant pollen in remote aerosol, deposited snow, and ice have been recently evaluated (Elster et al., 2007). As a product of this study cultivable pigmented colonies were obtained from alpine snow (Alps and Andes) and aerosol (Antarctic) samples.

It has been suggested (Imshsnetsky et al., 1978; Christner et al., 2000) that the presence of highly pigmented bacterial colonies in the mesosphere and in glacial ice was associated with the necessity to protect cells from harmful UV-radiation during atmospheric transport and exposure on the surface of the glaciers. Morphologically similar bacterial specimens have been also observed in soils in the Arctic Svalbard (Rehakova et al., 2008[1]). These observations support the idea that this type of microorganisms commonly develop in cold desert ecosystems and are easily transported via aerosol to both, short and long distances.

In this work we report the identification, using molecular ecology methodologies, of the microorganisms able to produce pigmented colonies in enrichment cultures of samples obtained from alpine snow (Alps and Andes) and aerosol (Antarctic). For comparison the culture of pigmented bacteria isolated from maritime Antarctic soil (King George Island, South Shetland) was also analysed.

2 Material and methods

2.1 Site description and sample collection

Aerosol samples originated from a set of filters collected between 1994 and 2000 at the coastal Antarctic Station Dumont d'Urville (66°40′ S, 140°01′ E). Samples were col-

[1] Rehakova, K., Brynychova, K., and Elster, J.: Diversity and abundance of soil algae along the East Brogger deglaciated moraine, Ny-Alesund, Svalbard, Polar Res., submitted, 2008.

lected on Gelman Zefluor® filters (47 mm diameter, 0.5 μm pore size) by drawing in air at a flow rate of 1.5 $m^3\,h^{-1}$. The sampling intervals were of 40 h, which corresponds to a volume between 30 and 60 m^3 of air.

In the year 2000, two European alpine snow pits were dug at very high elevation sites in the Mt. Blanc area of the Alps (0.6 m deep) at Col du Midi (elev. 3532 m, 17 May); and the other (2 m deep) at Col du Dome (elev. 4250 m, 31 August). Snow collected from recent deposits contained several visible dust layers. This dust is known to be transported by wind to the Alps from the Sahara desert (Oeschger et al., 1977; Wagenbach, 1989; De Angelis and Gaudichet, 1991). In addition, also in 2000, surface snow blocks were collected at the summit of Nevado Illimani, Bolivia (16°37′ S, 67°46′ W, Cordillera Real, elevation 6350 m) in the Andes. The Bolivian Andes are surrounded by the Altiplano, a high altitude desert (mean elevation: 3700 m a.s.l., Clapperton, 1993). This region, in particular, contains large salt flats, which are an important source of dust for the regional atmosphere during the dry season (Risacher, 1992). In the austral summer season of 2005, soil samples were collected in close vicinity of the Uruguay Antarctic Station Artigas, King George Island, South Shetlands.

All samples were collected using pre-cleaned glass vials (aerosol) and sealed plastic bags (snow and soil), respectively, transported frozen to the laboratory in Grenoble and/or in Tebo and stored frozen until further analysis (for more details see Elster et al., 2007).

2.2 Sample preparation and cultivation

To minimise possible contaminations, all post-sampling manipulations were done in a UV-sterilised laminar box hood, using sterile glass vials. To retrieve samples from the snow pits an upper layer of about 2 cm was sliced with a sharp knife. Snow samples were dried off at a temperature of −40°C in a special sterile lyophilisation device (Lyovac GT2, Leybold-Heraeus, Germany). After this operation, glass vessels containing solid deposits were rinsed with 10–15 ml of re-distilled water.

The aerosol filters were cut into quarters and one quarter was used for enrichment cultures. Each snow and aerosol sample was cultured in a sterile glass bottle (25 ml in volume). The bottom of each glass bottle was filled to a depth of one centimeter with glass beads (diameter 3 mm). BG-11 culture media (Bischoff and Bold, 1963) was used for all experiments. Each glass bottle was filled with about 10 ml of sterile medium (for more details see Elster et al., 2007). For soil samples analysis, suspensions of 10 g soil + 90 ml sterile water were homogenized using ultrasonication for 4 min and spread on Petri dishes containing mineral agar (0.1 ml of soil suspension per Petri dish with 1.5% BG11 agar in four replicates) (Elster et al., 1999). Glass bottles and Petri dishes were cultivated in an illuminated (~100 W cm^2) refrigerator (temperature 5–8°C) with a light regime of 18 h of light, 2 h of UV-B radiation (germicide lamp) and 4 h of darkness. The germicide lamp was used to sterilize the culture growth area (UV-B light did not penetrate through the glass bottles). Experimental bottles were shaken every 2–3 days. After 1 and 2 months of cultivation, the contents of bottles and Petri dishes were analyzed under the light microscope (Olympus BX 60). Selected samples with pigmented microbial colonies were analyzed by fluorescence after staining with the DAPI fluorochrome (EFM Olympus BX 60) (Zachleder and Cepák, 1987) and transmission electron microscopy (Jeol equipment JEM 1010).

Five pigmented microbial colonies were selected for further analysis (Fig. 1):

1. aerosol sample collected on 7 July 1999, in the coastal Antarctic Station Dumont d'Urville (66°40′ S, 140°01′ E).
2. Col du Midi (Alps) snow samples from a depth between 10 to 20 cm (elevation. 3532m, collected on 17 May 2000).
3. Col du Dome, Vallot (Alps) snow samples from surface to a depth of 10 cm (elevation. 4250 m, collected on 31 August 2000).
4. Nevado Illimani (Andes) surface snow blocks (16°37′ S, 67°46′ W, Cordillera Real, elevation 6350 m) collected in 2000.

5. Soil sample from the Uruguay Antarctic Station Artigas, King George Island, South Shetland Islands (62°10′ S, 58°30′ W), collected in the austral summer of 2005.

All cultures were transported to the Centro de Astrobiología (CAB) in Madrid, where microorganisms were identified by comparison of 16S rRNA gene sequences.

2.3 DNA extraction

One ml of each enrichment culture was used for DNA extraction. Fast DNA Spin kit for soil (Q-Bio Gene Inc., CA, USA) was used according to the manufacturer's instructions. To disrupt the cells, the mixture of ceramic and silica beads provided in the kit and three pulses of 40 s at speed 5.5 of the FastPrep bead-beating instrument (Bio 101) were applied. After the extraction, DNA was purified by passage through a GeneClean Turbo column (Q-Bio Gene Inc., CA, USA) and quantified by ethidium bromide-UV detection on an agarose gel (González-Toril et al., 2006).

2.4 16S ribosomal RNA clone library construction

PCR amplification of 16S rRNA gene fragments between *E. coli* positions 8 and 1507 for *Bacteria* domain (Lane, 1991), between *E. coli* position 25 and 1492 for *Archaea* domain (Achenbach, and Woese, 1995), and between *E. coli* position 359 and 805 for *Cyanobacteria* (Nübel et al., 1997) were performed. These genes were amplified by PCR in mixtures containing 20-30 ng of DNA per 50 μl reaction volume, 1× PCR buffer (Promega Biotech Iberica, Spain), 2.5 μM of each of the deoxynucleotides (Amersham Biosciences, UK), 2.5 mM $MgCl_2$, 1 mg mL^{-1} bovine serum albumin (BSA), 500 mM of each forward and reverse primers and 0.025 U/μl of Taq DNA polymerase (Promega Biotech Iberica, Spain) (Table 1). PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 40 s, annealing at 52°C for 1 min for the *Bacteria* domain, 56°C for the *Archaea* domain, and 60°C for the *Cyanobacteria*. Large 16S rRNA gene fragments (>1400 bp) were purified by

GeneClean Turbo Column (Q-Bio Gene Inc. CA, USA) and cloned using the Topo Ta Cloning Kit (Invitrogen. CA, USA). Cloned inserts were amplified using PCR conditions described above and they were directly sequenced with a Big-Dye sequencing kit (Applied Biosystem) following the manufacturer's instructions (González-Toril et al., 2006).

2.5 Clone library analysis

Sequences were analyzed using BLAST at the NCBI database (http://ncbi.nlm.nih.gov/BLAST) and added together with the most important BLAST hits, to an alignment of 50 000 homologous bacterial 16S rRNA primary structures by using the aligning tool of the ARB software package (http://www.arb-home.de) (Ludwig et al., 2004). The rRNA alignments were corrected manually and alignment uncertainties were omitted in the phylogenetic analysis. Phylogenetic trees were generated using parsimony, neighbour-joining, and maximum-likelihood analyses with a subset of 200 nearly full-length sequences (>1400 bp). Filters, which excluded highly variable positions, were used. In all cases, general tree topology and clusters were stable. A consensus tree was generated (González-Toril et al., 2006). Sequences obtained in this study have been deposited in the EMBL sequence database under accession numbers from EU429484 to EU429508.

3 Results and discussion

Following the protocols for enrichment cultures of photoautotrophic microorganisms present in snow samples from the Alps and the Andes and in an aerosol sample from Antarctica, different pigmented non-photosynthetic prokaryotes were able to grow in the extremely poor nutritional conditions of the selected media (Fig. 1) (Elster et al., 2007). Due to the characteristics of the cultures and the idiosyncrasy of the samples it was considered of interest to identify the microorganisms present in the different cultures, to

evaluate the diversity corresponding to this very specific type of microorganisms, and eventually to compare the results obtained in geographically dispersed sampling sites. A commercial soil DNA extraction kit was used to extract DNA from the enrichment cultures from particulate matter present in high elevation snow in the Alps and the Andes, from an Antarctic aerosol and a maritime Antarctic soil. DNA was extracted from all samples. No phototrophic *Bacteria* (cyanobacteria) were amplified using specific primers in any of the DNA extracted from the five samples. These results agree with the microscopy analysis of the cultures. Also no *Archaea* were detected by amplification.

PCR amplification of 16S rRNA genes using universal bacterial primers, followed by cloning and sequencing produced around 200 sequences which were used to identify the different bacteria present in the enrichment cultures by comparison with the NCBI database. The retrieved sequences were added to a database of over 50 000 prokaryotic 16S rRNA gene sequences using the aligning tool of the ARB software package (http://www.arb-home.de) (Ludwig et al., 2004). Phylogenetic trees were generated using parsimony, neighbour joining, and maximum-likelihood analyses with a subset of 200 nearly full-length sequences (>1400 bp). Filters excluding highly variable positions were used. In all cases general tree topology and clusters were stable. Thus, consensus trees were generated (Figs. 2–6).

After the analysis of the generated sequences representatives of three bacterial phyla: *Proteobacteria* (*Alpha*, *Beta* and *Gammaproteobacteria*), *Actinobacteria* and *Bacteroidetes*, were identified (Fig. 2). Seven close relative microorganisms have been identified for the snow sample from Col du Midi (Alps). Three sequences corresponded to the *Alphaproteobacteria*: one as a possible member of the genus *Agrobacterium* (closest relative: clone IrT-J614-14, retrieved from an environmental sample of a uranium mine (Selenska-Pobell, 2002)) (Fig. 3); the other two, close to clones B3NR69D12 and AP-12, detected in a underground cave (Northup et al., 2003) and in a marine estuary (NCBI accession number AY145551, unpublished) respectively. Two sequences corresponded to the *Betaproteobacteria*: one a possible member of the species *Hydrogenophaga palleronii* (Fig. 4), identified by FISH in a lake snow ag-

gregates (Schweitzer et al., 2001), and the other close to a clone of the genus *Limnobacter*, D-15, isolated from underground mineral water (Loy et al., 2005). One sequence corresponding to the *Gammaproteobacteria* exhibited a high homology with *Pseudomonas pseudoalkaligenes*, which was isolated from a marine sediment (NCBI accession number AF286035, unpublished). One sequence corresponded to the phylum *Bacteroidetes*, with high homology with *Aquiflexus balticus* (Fig. 5), also retrieved from a marine sediment (Brettar et al., 2004).

The other sample from the Alpes (Col du Dome) exhibited a much lower diversity than the one obtained from Col du Midi. In this sample only three sequences were retrieved. Two corresponded to uncultivated *Alphaproteobacteria* clones, AP-12 and O15B-H01, the first detected in a marine estuary (NCBI accession number AY145551, unpublished) and the other from ground water of a uranium mine (NCBI accession number AY662032, unpublished). The other sequence corresponded to the class *Actinobacteria*, with a high level of homology with the species *Dietzia kujamensis*, which was isolated from the Himalaya (Mayilraj et al., 2006).

Eight close relative microorganisms have been identified for the snow sample from the Andes (Nevado Illimani). Three sequences corresponded to the *Alphaproteobacteria* class: two related to the *Agrobacterium* (clone IrT-J614-14) (Fig. 3) and the clone B3NR69D12, both identified in Col du Midi; and one related to the uncultured EV818CFSSAHH29 clone, which has been identified in subsurface water of the Kalahari Shield in South Africa (NCBI accession number DQ336984, unpublished). Two sequences corresponded to the *Betaproteobacterai* class: *Hydrogenophaga palleronii* (Fig. 4) and *Limnobacter* clone D-15, both also identified in Col du Midi. One sequence exhibiting high homology with *Aquiflexus balticus* (Fig. 5) of the phylum *Bacteroidetes*, already described in Col du Midi, has been retrieved from the Andes enrichment culture. The last two sequences corresponded to the *Actinobacteria* class: one with high homology to *Microbacterium thalassium* (Richert, 2007) and the other with *Pseudonocardia* sp. (Fig. 6) which has been isolated from marine sediments (NCBI accession number AY974793, unpublished).

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Seven sequences retrieved from the aerosol sample from Antarctica were identified by phylogenetic analysis. Three sequences corresponded to the *Alphaproteobacteria* class: one related with clone B3NR69D12, which has been also identified in the Andes and the Alps (Col du Midi); another related with clone AP-12 which has been also identified in Col du Midi; and a third one corresponding to the *Brevundimonas vesicularis*, which was isolated from an ice core of Greenland (Sheridan et al., 2003). One sequence corresponded to the *Betaproteobacteria* and had a high level of homology with the uncultured clone of *Limnobacter* D-15, already identified in the enrichment cultures of snow samples from the Andes and the Alps (Col du Midi). Three sequences corresponded to the *Actinobacteria* class: one related with *Pseudonocardia antarctica* (Fig. 3), a psycchrophilic microorganisms isolated from McMurdo Valley in the Antarctica (Prabahar et al., 2004); a second one corresponding to a member of the *Pseudonocardia* genus (Fig. 6) isolated from marine sediments and also identified in the Andes; and a third one similar to *Brachybacterium conglomeratum*, which has been isolated from a spacecraft assembly facility (NCBI accession number AY145551, unpublished).

The sample from maritime Antarctica soil was a pure culture because only one sequence was retrieved with a high level of 16S rRNA gene sequence homology with a member of the *Brevundimonas* sp. identified previously in permafrost samples and close to the *Alphaproteobacteria* species of *Brevundimonas* identified in the Antarctic aerosol.

All the identified microorganisms in the enrichment cultures belong to the bacterial domain. Snow samples from the Alps (Col du Midi) and the Andes (Illimani), and the Antarctic aerosol sample exhibited a similar level of bacterial diversity in the corresponding enrichment cultures (Table 2). The maritime Antarctic soil (King George Island, Uruguay Antarctic Station Artigas) enrichment culture contained only one sequence (Table 2). This is probably due to the protocol used for its enrichment. Only one type of bacteria was able to grow on mineral agar plate. On the contrary, in the case of snow and aerosol samples growth in liquid mineral media was always observed. The

distribution of detected bacteria was related with cold environments (21.4%), marine environments (35.7%) and soils/subsurface (35.7%) (Table 2). No single cosmopolitan bacterium was found in all the analyzed sites, although three geographically distant sampling sites (Alps, Illimani and Antarctic aerosol) shared three microorganisms (*Limnobacter* clone D-15, and two uncultured *Alphaproteobacteria*, clones B3NR69D12 and AP-12). Different bacteria were found in two locations: *Agrobacterium* sp. (Col du Midi and Illimani), *Brevundimonas* sp. (Antarctic aerosol and maritime Antarctic soil), *Hydrogenophaga palleroni* (Col du Midi and Illimani), *Aquiflexus balticus* (Col du Midi and Illimani) and *Pseudonocardia* sp. (Illimani and Antarctic aerosol) (Table 2). Six out of fourteen identified genus or species are found only in a single location (Table 2). Interestingly enough the diversity found in very close locations in the Alps (Col du Dome and Col du Midi) are much more different (only one common sequence out of ten) than the diversity found in locations in different hemispheres, altitudes or matrix (snow versus aerosol) (Table 2). Concerning the pigments giving color to the microorganisms, several bacteria related with those present in the enrichment cultures have been described as having this peculiar phenotypic property, like *Dietzia*, *Aquiflexum*, *Microbacterium* and *Pseudonocardia*. In addition, members of the *Actinobacteria* class are well known by their ability to synthesize pigments for radiation protection purposes.

Concerning the different models for long distance microbial dissemination (atmospheric circulation, ocean currents, birds, fish, mammals and human vectors), if we consider the habitats in which related microorganisms have been detected, we can rule out ocean currents and animal vectors, leaving atmospheric circulation as the most plausible mean of dissemination, specially considering the microbial diversity detected in the Antarctic aerosol and shared with other distant locations (71.4%), which underlines the importance of microbes attached to dust particles for its disseminations.

Of course our data can not rule out the possible model of "everything is everywhere", although considering the experimental constraints introduced by the use of extremely oligotrophic selective media, we think that atmospheric dispersion can explain adequately the observed results. One of the problems related with this type of experiments

is to rule out the possibility of contamination. Considering the location of the selected sampling sites and the obtained results we think that this possibility is improbable. Although the difference observed between the samples obtained in the same campaign in two close sites in the Alps is not easy to explain, it is a very useful internal control of lack of contamination on the sampling manipulation. We strongly believe that the common microbial patterns of peculiar microorganisms observed at distant locations reflects truly airborne microbial dissemination, so we will like to propose this protocol for further testing this model of microbial dispersion.

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Table 1. Primers used for PCR amplification.

Primer[a]	Target site[b]	Sequence (5′ to 3′)	Specificity	Reference
8F	8–23	AGA GTT TGA TCM TGG C	*Bacteria* Domain	Lane (1991)
25F	9–25	CYG GTT GAT CCT GCC RG	*Archaea* Domain	Achenbach and Woese (1995)
1492R	1492–1513	TAC GGY TAC CTT GTT ACG ACT T	Universal	Achenbach and Woese (1995)
Cya359F	359–378	GGG GAA TYT TCC GCA ATG GG	*Cyanobacteria* and chloroplast	Nübel et al. (1997)
Cya106F	106–127	CGG ACG GGT GAG TAA CGC GTG A	*Cyanobacteria* and chloroplast	Nübel et al. (1997)
Cya781R(a)[c]	781-805	GAC TAC TGG GGT ATC TAA TCC CAT T	*Cyanobacteria* and chloroplast	Nübel et al. (1997)
Cya781R(b)[c]	781-805	GAC TAC AGG GGT ATC TAA TCC CTT T	*Cyanobacteria* and chloroplast	Nübel et al. (1997)

[a] F (foward) and R (reverse) indicate the orientations of the primers in relation to the rRNA.
[b] Positions are given according to the *E. coli* numbering of Brosius et al. (1981).
[c] Reverse primer CYA781R is an equimolar mixture of CYA781R(a) and CYA781R(b).

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Table 2. Closet phylogenetic relatives. Microorganisms and sequences closely related with clones obtained in this studies and some of their characteristics.

Col du Dome (Alps)	Col du Midi (Alps)	Illimani (Andes)	Artigas (Antarctic)	aerosol (Antarctic)	Interesting characteristics	NCBI[a]
Clone AP-12 Near *Bradyrhizobium* *Alphaproteobacteria*	Clone AP-12 Near *Bradyrhizobium* *Alphaproteobacteria*	—	—	Clone AP-12 Near *Bradyrhizobium* *Alphaproteobacteria*	Uncultured bacterium from Weser Estuary	AY145551
—	Clone B3NR69D12 Near *Afipia massilien-sis* *Alphaproteobacteria*	Clone B3NR69D12 Near *Afipia massiliensis* *Alphaproteobacteria*	—	—	Un cultured bacterium from an inhabiting ferroman-ganese deposits in Lechuguilla and Spider Caves	AY186080
Clone O15B-H01 Near *Sinella granuli Al-phaproteobacteria*	—	—	—	—	Uncultured bacterium from a groundwater contaminated with high levels of nitric acid-bearing uranium water	AY662032
—	Clone IrT-J614-14 Near *Agrobacterium* *Alphaproteobacteria*	Clone IrT-J614-14 Near *Agrobacterium* *Alphaproteobacteria*	—	—	Uncultured bacterium from a uranium mining waste piles	AJ295675
—	—	Clone EV818CFSSHH29 Near *Brevundimonas* *Alphaproteobacteria*	*Brevundimonas* sp. *Alphaproteobacteria*	*Brevundimonas vesicu-laris* *Alphaproteobacteria*	– Uncultured bacterium from subsurface water of Kala-hari Shield, South Africa – Bacteria isolated from permafrost. – Anaerobic psychrophilic enrichment cultures obtained from a Greenland glacier ice core – Bacteria detected in lake snow aggregates by FISH	DQ336984 DQ177489 AY169433
—	Clone D-15 Near *Limnobacter* *Betaproteobacteria*	Clone D-15 Near *Limnobacter* *Betaproteobacteria*	—	Clone D-15 Near *Limnobacter* *Betaproteobacteria*	Uncultured bacterium from natural mineral water	AF522999
—	*Hydrogenophaga* *palleronii* *Betaproteobacteria*	*Hydrogenophaga* *palleronii* *Betaproteobacteria*	—	—	Bacteria detected in lake snow aggregates by FISH	AF078769
—	Clone LCP-79 *Pseudomonas* *Gammaproteobacteria*	—	—	—	Uncultured bacterium from marine sediments	AF286035
Dietzia spp. *Actinobacteria*	—	—	—	—	Bacteria isolated in cold environments (Himalaya and ar-tic Ocean)	DQ060378 AY972480 AB159036
—	—	*Pseudonocardia* sp. *Actinobacteria*	—	*Pseudonocardia antarc-tica* *Actinobacteria*	– Bacteria isolated from marine sediments (depths of 500 m). – Bacteria isolated from McMurdo Dry Valleys, Antarc-tica. Filamentous and produce brown colour substrate mycelia and aerial mycelia Vich form a white conglom-erate.	AY974793 DQ448725 AJ576010
—	—	*Microbacterium thalas-sium* *Actinobacteria*	—	—	In young cultures, cells are small irregular rods. In old cultures, rods become shorter or spherical elements. Colonies are yellowish while, yellow or orange.	AM181507
—	—	—	—	*Brachybacterium* sp. *Actinobacteria*	Bacteria isolated from spacecraft assembly facilities	AY167842
—	*Aquiflexum balticum* *Bacteroidetes*	*Aquiflexum balticum* *Bacteroidetes*	—	—	Bacterium isolated from surface water of the Central Baltic Sea (depth of 5 m). Red and transparent colonies when young, but turn opaque with ongoing incubation. Cells contain carotenoids.	AJ744861

[a] Accession Number in NCBI of the closest phylogenetic relatives

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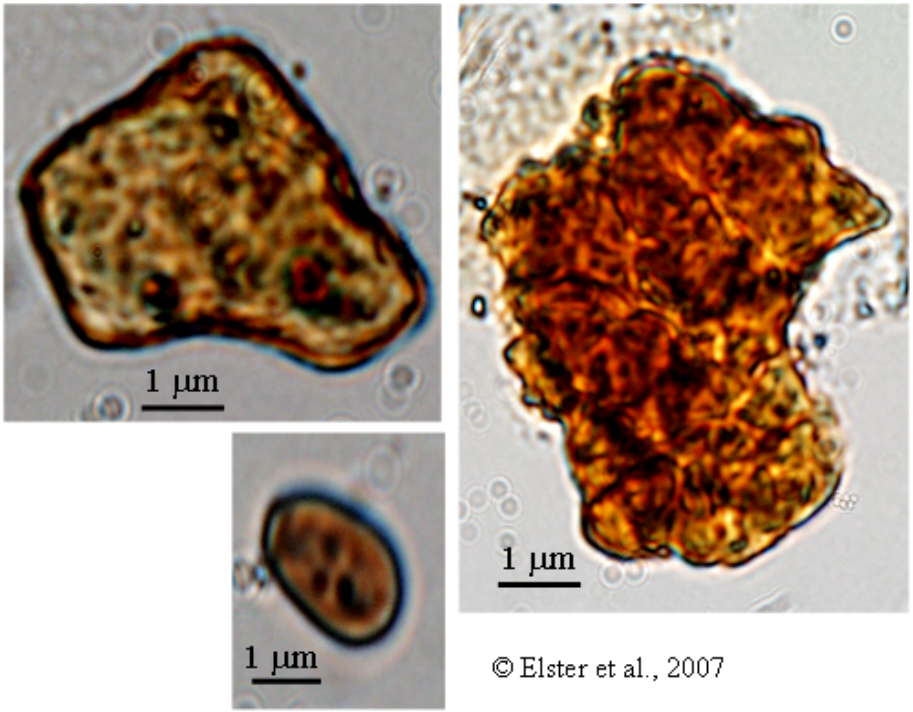

Fig. 1. Display of pigmented microorganisms enriched from snow and aerosol samples.

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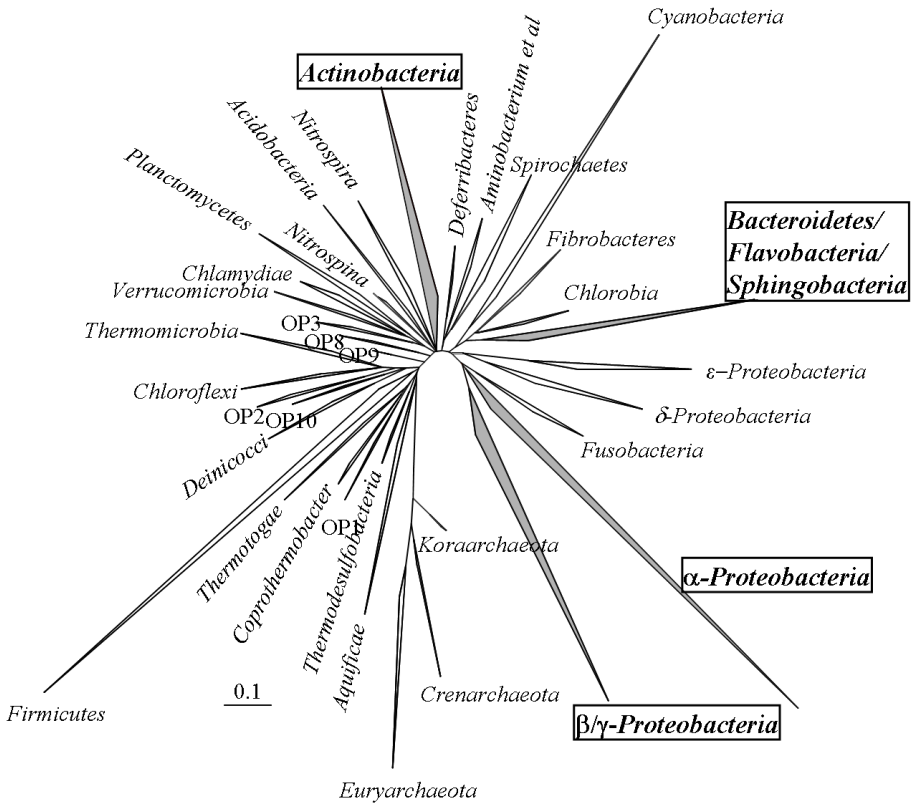

Fig. 2. Prokaryotic phylogenetic tree showing in red the phyla in which pigmented microorganisms have been identified.

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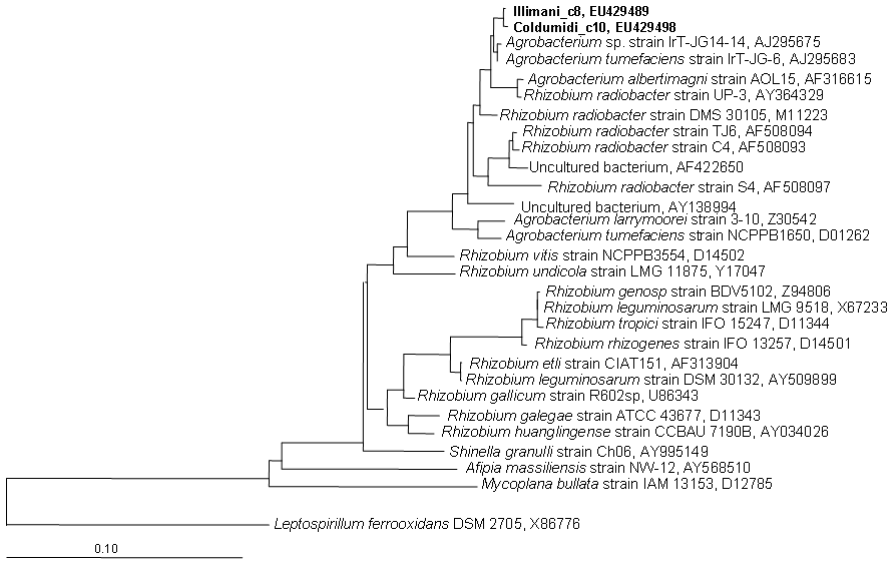

Fig. 3. Phylogenetic tree of *Agrobacterium* and related bacteria. Phylogenetic trees were generated using parsimony, neighbor-joining and maximum likelihood analysis with different sets of filters. All generated phylogenetic trees resulted in stable branching. A consensus tree was generated using all calculated trees. Cloned 16S rRNA sequences from enrichment cultures are indicated in bold. Sequences retrieved from Nevado Illimani (Andes) are designated by the word "Illimani" followed by the number of the sequence. Sequences from Col du Midi (Alps) are designated by "Coldumidi" followed by the number of the sequence. Bar represents 10% estimated phylogenetic divergence.

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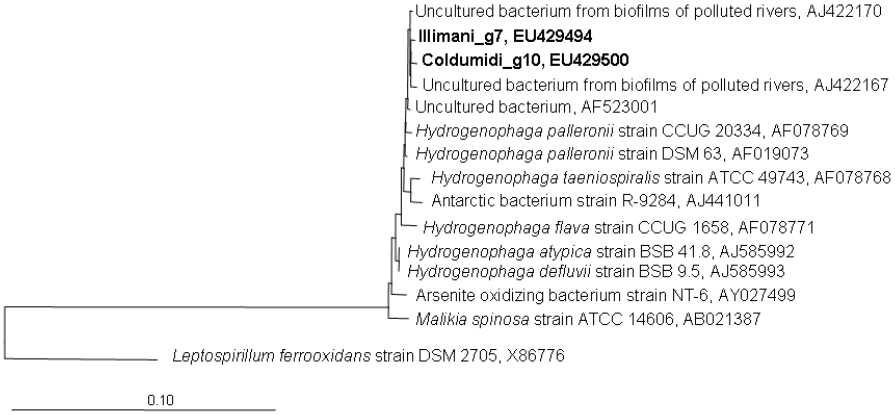

Fig. 4. Phylogenetic tree of *Hydrogenophaga* and related bacteria. Phylogenetic trees were generated using parsimony, neighbor-joining and maximum likelihood analysis with different sets of filters. All generated phylogenetic trees resulted in stable branching. A consensus tree was generated using all calculated trees. Cloned 16S rRNA sequences from enrichment cultures are indicated in bold. Sequences retrieved from Nevado Illimani (Andes) are designated by the word "Illimani" followed by the number of the sequence. Sequences from Col du Midi (Alps) are designated by "Coldumidi" followed by the number of the sequence. Bar represents 10% estimated phylogenetic divergence.

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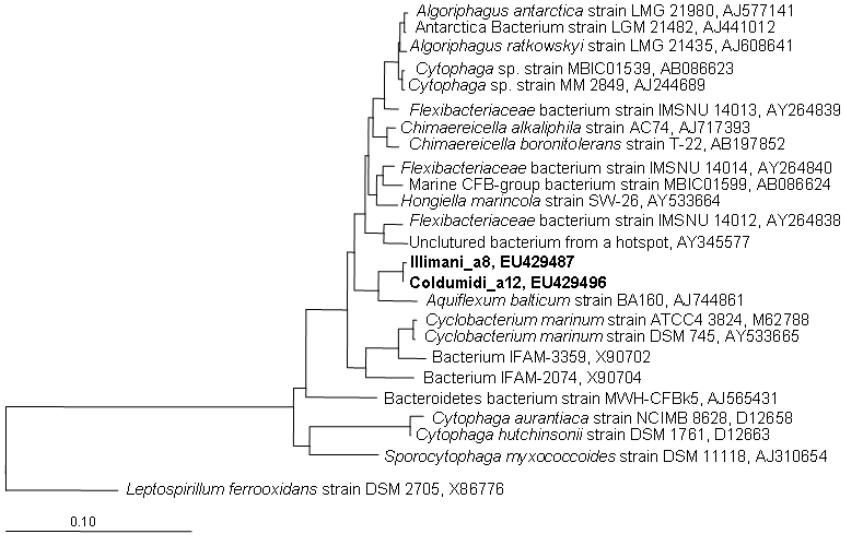

Fig. 5. Phylogenetic tree of *Aquiflexus* and related bacteria. Phylogenetic trees were generated using parsimony, neighbor-joining and maximum likelihood analysis with different sets of filters. All generated phylogenetic trees resulted in stable branching. A consensus tree was generated using all calculated trees. Cloned 16S rRNA sequences from enrichment cultures are indicated in bold. Sequences retrieved from Nevado Illimani (Andes) are designated by the word "Illimani" followed by the number of the sequence. Sequences from Col du Midi (Alps) are designated by "Coldumidi" followed by the number of the sequence. Bar represents 10% estimated phylogenetic divergence.

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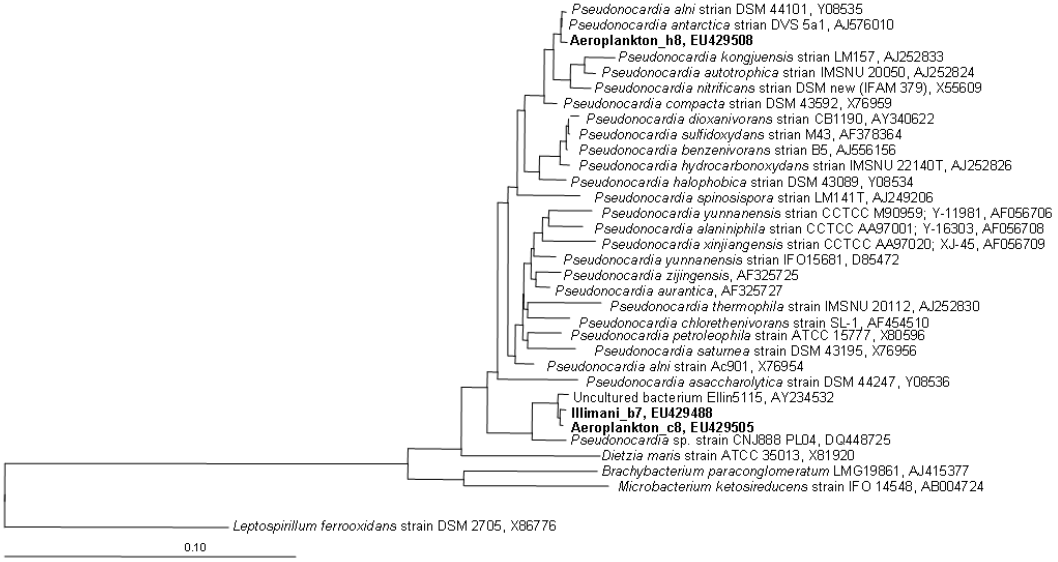

Fig. 6. Phylogenetic tree of *Pseudonocardia* and related bacteria. Phylogenetic trees were generated using parsimony, neighbor-joining and maximum likelihood analysis with different sets of filters. All generated phylogenetic trees resulted in stable branching. A consensus tree was generated using all calculated trees. Cloned 16S rRNA sequences from enrichment cultures are indicated in bold. Sequences retrieved from Nevado Illimani (Andes) are designated by the word "Illimani" followed by the number of the sequence. Sequences from aerosol sample recollected in Antarctica are designated by "Aeroplankton" followed by the number of the sequence. Bar represents 10% estimated phylogenetic divergence. Additional figures can be viewed in the supplementary material (http://www.biogeosciences-discuss.net/5/1607/2008/bgd-5-1607-2008-supplement.pdf).

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